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     (FILE 'HOME' ENTERED AT 14:46:02 ON 02 JUN 2003)

     FILE 'MEDLINE' ENTERED AT 14:46:10 ON 02 JUN 2003
L1         14297 S LEISHMANIA?
L2          9896 S GM-CSF
L3            30 S L1 AND L2
L4        234336 S TRANSFECT? OR PLASMID? OR VECTOR?
L5          1825 S L1 AND L4
L6          1123 S L1(P)L4
L7           370 S L1(3A)L4
L8        207846 S TRANSFECT? OR PLASMID? OR HETEROLOGOUS
L9           419 S L1 AND L8

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=> D BIB AB 198

L9 ANSWER 198 OF 419 MEDLINE
AN 1998078996 MEDLINE
DN 98078996 PubMed ID: 9419187
TI Protection against **Leishmania** major challenge infection in mice vaccinated with live recombinant parasites expressing a cytotoxic gene.
AU Muyombwe A; Olivier M; Harvie P; Bergeron M G; Ouellette M; Papadopoulou B
CS Centre Hospitalier de l'Universite Laval et Departement de Microbiologie, Universite Laval, Quebec, Canada.
SO JOURNAL OF INFECTIOUS DISEASES, (1998 Jan) 177 (1) 188-95.
Journal code: 0413675. ISSN: 0022-1899.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199801
ED Entered STN: 19980206
Last Updated on STN: 19980206
Entered Medline: 19980123
AB A "suicide" system based on thymidine kinase-ganciclovir combination was developed and tested in a **Leishmania** major experimental model. Susceptible BALB/c mice were infected with L. major expressing the thymidine kinase gene of herpes simplex virus type 1 and treated for 2 consecutive weeks with 7.5 mg/kg/day ganciclovir at different times from the initial infection. Ganciclovir treatment at varying times after infection had different effects on the outcome of disease. A complete inhibition of intracellular parasites was obtained in mice treated 1 or 4 days after infection, whereas ganciclovir administration 2 weeks later resulted in the control of infection only when the drug was provided. Variable levels of protection, from partial to total, against challenge infection with virulent L. major were observed, depending on the timing of ganciclovir treatment. The thymidine kinase-ganciclovir approach represents an excellent experimental model to control **Leishmania** infection and to evaluate the immunologic response of the host.

INVENTOR

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L9 ANSWER 322 OF 419 MEDLINE
AN 93267108 MEDLINE
DN 93267108 PubMed ID: 8098724
TI **Transfected Leishmania** expressing biologically active IFN-gamma.
AU Tobin J F; Reiner S L; Hatam F; Zheng S; Leptak C L; Wirth D F; Locksley R M
CS Department of Tropical Public Health, Harvard School of Public Health, Boston, MA 02115.
NC AI 21365 (NIAID)
AI 26918 (NIAID)
SO JOURNAL OF IMMUNOLOGY, (1993 Jun 1) 150 (11) 5059-69.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199306
ED Entered STN: 19930702
Last Updated on STN: 19950206
Entered Medline: 19930622
AB Infection of susceptible BALB/c mice with **Leishmania** major leads to progressive infection with the failure to expand and activate Th1 CD4+ T cells that elaborate IFN-gamma, a critically implicated cytokine for control of disease. We used the recently described capacity to express foreign genes in trypanosomatids to introduce into **Leishmania** the murine IFN-gamma gene on a drug-selectable **plasmid** under the constitutive control of intergenic tubulin sequences. Several clones of L. major were established and demonstrated to contain IFN-gamma DNA and IFN-gamma RNA that was appropriately trans-spliced with the **Leishmania**-specific leader sequence, and to secrete IFN-gamma into the media. The secreted IFN-gamma was biologically active as assessed by up-regulation of class II MHC Ag and induction of macrophage nitric oxide synthase activity in a macrophage cell line. Infection of nude mice with IFN-gamma-containing organisms resulted in significantly slower progression of disease as compared to infection with organisms containing the empty **plasmid**, suggesting that biologically important activation of infected macrophages might be occurring in vivo. Infection of genetically susceptible BALB/c mice, however, did not impede the expansion of Th2 cells and the inexorable progression of disease. Despite the demonstration of increased levels of IFN-gamma transcription in vivo, induction of nitric oxide synthase in macrophages and expression of Ly-6, and IFN-gamma-inducible Ag, on CD4+ lymphocytes could not be shown. In all cases, organisms recovered from tissue amastigotes contained the IFN-gamma **plasmid** and secreted active IFN-gamma. The data confirm earlier studies that IFN-gamma alone is not sufficient to impede activation and maturation of Th2 cells in susceptible mice, even when targeted directly to the infected cell.

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teaching gets against invention for cytokine transfection in general

L9 ANSWER 378 OF 419 MEDLINE
AN 88309783 MEDLINE
DN 88309783 PubMed ID: 2841973
TI **Heterologous** expression of the bifunctional thymidylate synthase-dihydrofolate reductase from **Leishmania** major.
AU Grumont R; Sirawaraporn W; Santi D V
CS Department of Biochemistry and Biophysics, University of California, San Francisco 94143.
NC AI19358 (NIAID)
SO BIOCHEMISTRY, (1988 May 17) 27 (10) 3776-84.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198810
ED Entered STN: 19900308
Last Updated on STN: 19980206
Entered Medline: 19881011
AB The bifunctional thymidylate synthase-dihydrofolate reductase (TS-DHFR) of **Leishmania** major has been cloned and expressed in Escherichia coli and Saccharomyces cerevisiae. The strategy involved placing the entire 1560-bp coding sequence into a parent cloning **plasmid** that was designed to permit introduction of unique restriction sites at the 5'- and 3'-ends. In this manner, the entire coding sequence could be easily subcloned into a variety of expression vectors. High levels of TS-DHFR gene expression were driven by tac, pL and T7 RNA pol promoters in E. coli, and the GAPDH-ADH-2 promoter in S. cerevisiae. L. major TS-DHFR also complemented TS deficiency in E. coli. In E. coli, the protein accumulated to very high levels, but most was present as inactive inclusion bodies. Nevertheless, substantial amounts were soluble; up to 2% of the soluble protein was catalytically active TS-DHFR. In the yeast systems, essentially all of the bifunctional protein was soluble and catalytically active, and crude extracts contained about 100-fold more enzyme than do extracts from wild-type L. major. The expressed TS-DHFR from yeast and E. coli was purified to homogeneity by methotrexate-Sepharose affinity chromatography. About 8.5 mg of homogeneous, catalytically active protein is obtained from a 1-L culture of yeast, and 1.5 mg was obtained from 1 L of E. coli culture. A 200-L fermentation of the yeast expression system yielded a crude extract containing over 4 g of TS-DHFR.(ABSTRACT TRUNCATED AT 250 WORDS)

L9 ANSWER 369 OF 419 MEDLINE
AN 90083219 MEDLINE
DN 90083219 PubMed ID: 2594753
TI **Transfection** of **Leishmania** enriettii and expression of chloramphenicol acetyltransferase gene.
AU Laban A; Wirth D F
CS Department of Tropical Public Health, Harvard School of Public Health, Boston, MA 02115.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1989 Dec) 86 (23) 9119-23.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199001
ED Entered STN: 19900328
Last Updated on STN: 19980206
Entered Medline: 19900119
AB We report a transient expression **transfection** system in **Leishmania** enriettii. A hybrid gene containing an intergenic region of the alpha-tubulin cluster and the bacterial chloramphenicol

acetyltransferase (CAT; EC 2.3.1.28) gene is expressed after **transfection** of L. enriettii with the hybrid **plasmid**. The expression of the CAT gene is dependent on the presence of sequences from the alpha-tubulin gene. The hybrid gene is also active in **Leishmania** braziliensis and **Leishmania** major.

L9 ANSWER 368 OF 419 MEDLINE
AN 90136974 MEDLINE
DN 90136974 PubMed ID: 2300209
TI Stable expression of the bacterial neor gene in **Leishmania** enriettii.
AU Laban A; Tobin J F; Curotto de Lafaille M A; Wirth D F
CS Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts 02115.
SO NATURE, (1990 Feb 8) 343 (6258) 572-4.
Journal code: 0410462. ISSN: 0028-0836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199003
ED Entered STN: 19900328
Last Updated on STN: 19900328
Entered Medline: 19900313
AB Molecular genetic studies in parasitic protozoa have been hindered by the lack of methods for the introduction and expression of modified or foreign genes in these organisms. Two recent reports described the transient expression of the bacterial chloramphenicol acetyl transferase (CAT) gene under the control of parasite-specific sequences. We now describe the stable expression of a selectable marker, the gene for neomycin resistance (neor) in **Leishmania** enriettii. A chimaeric gene containing the neor gene inserted between two alpha-tubulin intergenic sequences was introduced into the cells and drug-resistant L. enriettii were observed which stably expressed the neor gene. One goal of this work was to analyse the sequences necessary for trans-splicing of messenger RNA, as trypanosomatids have a novel process of RNA trans-splicing, described initially in Trypanosome brucei and subsequently in several other trypanosomatids, including L. enriettii. Many trypanosomatid genes are arranged in tandem arrays and the intergenic sequences contain both the splice acceptor site for the addition of the spliced leader sequence and a putative polyadenylation site. Messenger RNA isolated from several different neor L. enrietti lines contained the spliced leader sequence joined to the neor gene at the position of the splice acceptor site in the alpha-tubulin intergenic sequence. The neor mRNA was also polyadenylated. **Plasmid** DNA is present within the drug-resistant organisms and appears to be extrachromosomal. The development of these methods allows the functional analysis of sequences necessary for trans-splicing.

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L9 ANSWER 353 OF 419 MEDLINE
AN 91304515 MEDLINE
DN 91304515 PubMed ID: 1906580
TI Stable DNA **transfection** of a wide range of trypanosomatids.
AU Coburn C M; Otteman K M; McNeely T; Turco S J; Beverley S M
CS Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115.
NC AI-20941 (NIAID)
AI-21903 (NIAID)
GM07196 (NIGMS)
SO MOLECULAR AND BIOCHEMICAL PARASITOLOGY, (1991 May) 46 (1) 169-79.
Journal code: 8006324. ISSN: 0166-6851.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199108
ED Entered STN: 19910908
Last Updated on STN: 19910908
Entered Medline: 19910821
AB We have shown that the **Leishmania** major **transfection** vector pR-NEO (or derivatives thereof) can be introduced and stably maintained in four species complexes of pathogenic **Leishmania** (L. tropica, L. mexicana, L. donovani, L. braziliensis), and the genera Endotrypanum and Crithidia; **transfection** of Trypanosoma cruzi or Trypanosoma brucei was not successful. Quantitative plating assays showed that the **transfection** efficiencies were high in L. major and **Leishmania** amazonensis (5x10(-5)/cell) and about 10-fold less for **Leishmania** panamaensis and Crithidia. **Leishmania** donovani **transfected** with pR-NEO retained the ability to infect hamsters, and amastigotes recovered after 2 months yielded G418-resistant promastigotes which retained high levels of extrachromosomal pR-NEO DNA. In promastigotes, the **transfected** DNA existed as extrachromosomal circles, and expressed the predicted 2.4-kb hybrid NEO/DHFR-TS mRNA bearing the trans-spliced miniexon. Large quantitative differences were observed only in Crithidia: relative to **transfected Leishmania** species, the copy number of pR-NEO was elevated 20-fold, while the levels of the NEO/DHRFR-TS mRNA or Escherichia coli beta-galactosidase (synthesized from the expression vector pX-beta GAL) were reduced 80 and more than 1000-fold, respectively. Thus, genetic signals derived from L. major DNA that mediate RNA expression or stability are recognized by the **heterologous Leishmania** species but less efficiently by Crithidia. These studies suggest that pR-NEO derived vectors may be applied to the study of genes expressed throughout the life cycle in a wide range of pathogenic trypanosomatids.

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L9 ANSWER 407 OF 419 MEDLINE
AN 84142075 MEDLINE
DN 84142075 PubMed ID: 6321982
TI Isolation and characterization of an alpha-tubulin gene from **Leishmania** enriettii.
AU Wirth D F; Slater C
SO MOLECULAR AND BIOCHEMICAL PARASITOLOGY, (1983 Sep) 9 (1) 83-92. Journal code: 8006324. ISSN: 0166-6851.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198404
ED Entered STN: 19900319
Last Updated on STN: 19900319
Entered Medline: 19840417
AB An alpha-tubulin gene of **Leishmania** enriettii has been identified in genomic Southern blots by hybridization with a **heterologous** alpha-tubulin gene from Drosophila melanogaster. A clone containing this gene has been isolated from a **plasmid** library of size-selected L. enriettii DNA. It was identified by hybridization with the D. melanogaster tubulin gene. The cloned DNA fragment was characterized by restriction analysis and partial DNA sequence analysis. The cloned DNA fragment is 2 kb in length, bounded by Pst I sites, and appears to contain the entire coding region of the alpha-tubulin gene.

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